Technical methods

Economic cultivation of "thermophilic" *Campylobacter* spp

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Optimum atmospheres for the growth of "thermophilic" *Campylobacter* spp contain between 5% and 10% oxygen and between 1% and 10% carbon dioxide, although concentrations slightly above or below these limits are acceptable in practice.¹ The necessary conditions are usually provided in a closed jar by replacing some of the air with gas from a cylinder of carbon dioxide with either hydrogen or nitrogen, or by using a hydrogen and carbon dioxide generating envelope and catalyst. In many countries, however, resources do not permit the use of these methods, and a candle jar is used instead. Burning a candle in a closed jar leaves 1.2-1.5% carbon dioxide and between 17.0% and 18.5% oxygen, an insufficient reduction of oxygen for some species of *Campylobacter*,² so a search has been made for a more effective and inexpensive alternative.³ Methylated spirit meets these requirements. Favourable conditions can be achieved in a jar without gas cylinders or generators simply by drawing a partial vacuum and supplying carbon dioxide.

Material and methods

STUDIES OF JAR ATMOSPHERE AFTER BURNING METHYLATED SPIRIT

A hole was drilled in the lid of a plastic 2.5 l anaerobic jar (BBL) to admit a Clark's oxygen electrode, a thermocouple thermometer, and a tap for registering pressure or drawing a vacuum. The probes and lid were protected against heat by a piece of aluminium foil on top of the plate carrier. In trials of the combustion of methylated spirit 0.5 ml was placed in a 40 mm glass petri dish resting on the top culture plate and ignited, and the lid of the jar applied after three seconds. The time was chosen as the safe maximum for the interchange of gas between plates and jar, production of carbon dioxide, and heating of the atmosphere in the jar before the

flame decreased. The heating produced a partial vacuum after the jar was sealed and cooled. Recordings were made, varying the number of culture plates between two and 10. The oxygen tensions before and after release of the vacuum were noted (after return to ambient temperature).

GROWTH OF CAMPYLOBACTERS IN VARIOUS ATMOSPHERES

Observations on the growth of campylobacters were made on the species *C jejuni* (NCTC 11168), *C coli* (NCTC 11353), and *C laridis* (NCTC 11352). The bacteria were grown in Preston broth at 42°C for 48 hours and portions then frozen at -70°C pending use. 20 µl volumes of tenfold dilutions were inoculated on to blood agar plates and incubated at 37°C for 24 hours; six plates were used for each jar.

In addition to comparisons between candle burns and methylated spirit combustion, other jars were depleted of oxygen by drawing a vacuum of 380 mm Hg and introducing a supplement of carbon dioxide. Briefly, the methods used were as follows: conventional candle jar; methylated spirit combustion as described above; drawing the vacuum and then tilting the jar to allow water to flow over 1 g citric acid and 1 g sodium bicarbonate in a dish to generate carbon dioxide; applying the vacuum to a conventional candle jar after the flame had died; drawing the vacuum, releasing it by admitting expired air from a bag, and finally, repeating the evacuation.

Results

MEASUREMENT OF ATMOSPHERES AFTER THE COMBUSTION OF METHYLATED SPIRIT

The combustion of methylated spirit reduced the concentration of oxygen from 21% to between 10.5% and 14.0% according to the loading of the jar (Table 1). When the vacuum produced in the jars by cooling the hot gas was released there was an instantaneous rise in the oxygen concentration before the probe could adjust to the admitted air. This allowed the effects of the vacuum and of the combustion itself to be distinguished (Table 1).

GROWTH OF CAMPYLOBACTERS IN EXPERIMENTAL ATMOSPHERES

Table 2 shows that the incubation of campylobacters in a candle jar is greatly inferior to incubation in jars prepared by the combustion of methylated spirit or by drawing a vacuum and providing a supplement of

Table 1 Depletion of oxygen by combustion of methylated spirit in jars containing varying numbers of culture plates

	No of plates				
	2	4	6	8	10
Oxygen lost by heating (%)	3.8	3.6	3.7	3.1	2.4
Oxygen lost by burning (%)	6.7	6.7	5.8	6.0	4.6
Final concentration of oxygen (%)	10.5	10.7	11.5	11.9	14.0

Table 2 Growth of three campylobacter species in jars prepared in different ways

	Log ¹⁰ values of colony counts with given jar preparation				
	Candle burn	Methylated spirit burn	Vacuum* plus added carbon dioxide		
			(a)	(b)	(c)
<i>C. laridis</i>	No growth	6.8	6.7	6.6	6.6
<i>C. coli</i>	4.7	6.0	5.7	6.5	6.2
<i>C. jejuni</i>	5.7	7.4	7.0	7.7	7.3

*380 mm Hg.

(a) by reaction of citric acid with sodium bicarbonate.

(b) by candle burn before drawing vacuum.

(c) by expired air.

carbon dioxide. Even more striking than the improvement in viable counts by the use of these simple alternatives to the candle jar was the much greater size of the colonies obtained.

Discussion

The combustion of methylated spirit provides an atmosphere much more suitable for the growth of "thermophilic" campylobacters than a candle jar, and with little extra trouble or expense. Some variation in the level of oxygen is inevitable according to the loading of the jar, but if this does not exceed eight plates in a standard anaerobic jar satisfactory results can be expected.

If a jar with a tap and means of drawing a vacuum are available levels of oxygen can be controlled

exactly, irrespective of loading. Three equally effective ways of providing the necessary supplement of carbon dioxide have been described.

References

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An easy way to orientate small muscle biopsy tissue

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With the advent of various histochemical techniques for studying human skeletal muscle the diagnostic value of muscle biopsy to help establish the major categories of muscle diseases has grown considerably. Undoubtedly, an open surgical biopsy can show a greater area of the diseased muscle and with

better precision. Percutaneous needle biopsy of skeletal muscle, on the other hand, is rapid and relatively atraumatic: complications are rare. In addition, minimal preparation of the patient is required, and biopsy can be carried out in the outpatient clinic as well as on the hospital ward. Specimens obtained by means of the needle biopsy technique, however, are usually small, which makes them difficult to orientate adequately for cutting true transverse sections. I describe a method that enables well orientated transverse cryostat sections to be obtained easily and essentially free of ice crystal artefacts.

Material and methods

The various pieces of muscle tissue obtained by percutaneous needle biopsy were placed on to a blank